

Biomimetic Design of a Microfluidic Device for Auto-separation of Leukocytes from Whole Blood

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ABSTRACT

An important application of microanalytical lab-on-a-chip devices is the analysis of leukocytes or their genetic material, for which these cells must first be isolated from the whole blood sample. We created a simple microfluidic device that takes advantage of the intrinsic features of blood flow in the microcirculation, such as plasma skimming and leukocyte margination, to separate leukocytes directly from whole blood. It operates on microliter samples of whole blood, provides positive, continuous flow selection of leukocytes and requires no preliminary labeling of cells. A single pass through the device produces a 34-fold enrichment of the leukocyte-to-erythrocyte ratio. This effortless, efficient and inexpensive technology can be used as a lab-on-a-chip component for initial whole blood sample preparation. Its integration into microanalytical devices that require leukocyte enrichment will enable accelerated transition of these devices into the field for point-of-care clinical testing.

Keywords: blood, leukocytes, margination, sample, preparation

1 INTRODUCTION

The rapidly expanding field of lab-on-a-chip microanalytical devices promises inexpensive, portable, miniaturized tools that could potentially revolutionize clinical and basic research analyses by dramatically reducing sample size and handling. An important application of this technology is the analysis of leukocytes (white blood cells) or their contents, for which these cells must first be isolated from the whole blood sample.

Human blood is a 45% suspension of cells (erythrocytes, platelets and leukocytes) in plasma. Erythrocytes (red blood cells) constitute the vast majority of all blood cells and are normally ~1000 times more abundant than leukocytes. Traditionally, several milliliters of whole blood are drawn and then centrifuged in order to separate blood cells of different density. Plasma, platelets, white and red cells can be separated by this procedure, but it is a labor-, energy- and time-intensive process that relies on special equipment and requires trained personnel. Many current microanalytical devices have no integrated sample

preparation capabilities and continue to rely on this conventional off-the-chip technique. Currently available methods that can be used for on-the-chip enrichment of leukocytes from whole blood are often complicated, lack versatility, offer low efficiency or require preliminary modification of cells [1,3,4,5]. Thus development of simple and efficient methods to isolate leukocytes directly from whole blood remains an important task.

Because of its particulate nature, blood exhibits unique flow characteristics on the scale of the microcirculation [6]. Erythrocytes, which are smaller and more deformable than leukocytes, tend to flow at the center of blood vessels, leaving a plasma-rich zone adjacent to the vessel wall [7]; they also flow faster than leukocytes [8]. This encourages mechanical interactions (collisions) between leukocytes and erythrocytes that influence the leukocyte distribution across the vessel lumen [8,9] and among vessels of the microcirculation *in vivo* [10,11]. In vessels larger than ~30 μm in diameter, collisions with passing erythrocytes force leukocytes to migrate toward the vessel wall in a process known as margination [8,12,13]. The tendency of erythrocytes to concentrate at the center of the blood stream also leads to plasma skimming – an uneven distribution of erythrocytes and plasma among the two daughter vessels of any nonsymmetrical bifurcation [14]: the vessel that receives less flow receives a disproportionately smaller fraction of erythrocytes, and therefore has a lower hematocrit.

Here, we describe the operation principle and demonstrate the efficiency of a simple microfluidic device that takes advantage of these natural flow properties of blood to separate leukocytes from whole blood. It consists of a network of rectangular microchannels of specific geometry and connectivity and requires only a small pressure gradient to produce a 34-fold enrichment of the leukocyte-to-erythrocyte ratio in a single pass. This novel leukocyte enrichment technique could be used to design custom lab-on-a-chip components for integrated sample preparation in microanalytical devices that require leukocytes, their DNA or RNA [1,3,4].

2 METHODS

Fabrication of microchannel networks and the experimental setup were previously described in detail

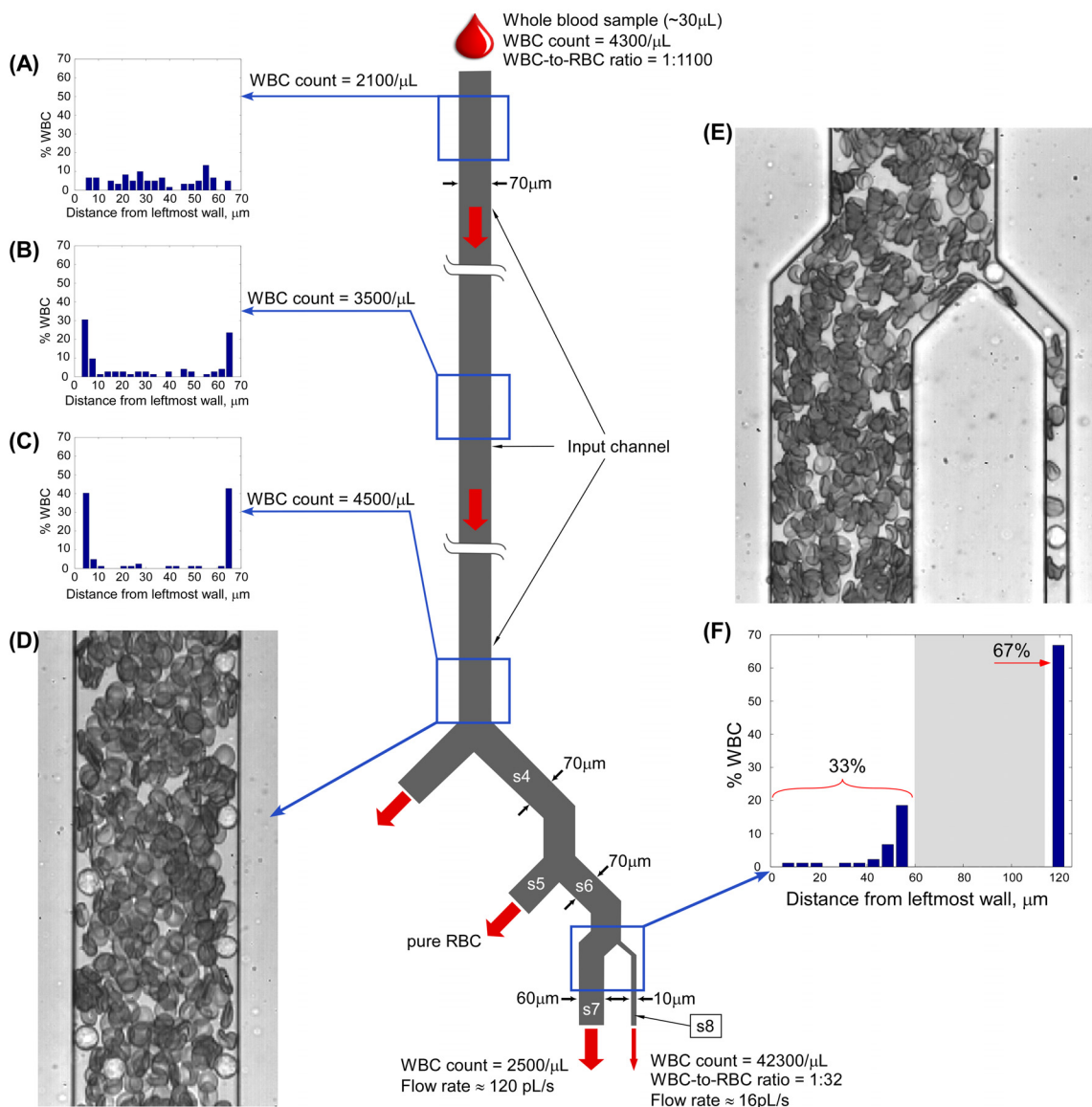


Figure 1. Central panel shows a schematic of the leukocyte separation device. Red arrows indicate direction of blood flow. All microchannels are $10.3\ \mu\text{m}$ -deep. The input channel is $\sim 5.5\text{-mm}$ -long; distance between the first (b1) and the third (b3) bifurcation is $0.48\ \text{mm}$. Panels (A), (B), and (C) show leukocyte distributions across the input channel width at different points along the channel. Panels (D) and (E) show representative images of the corresponding parts of the device; red blood cells appear dark. Panel (D) illustrates margination of leukocytes achieved along the input channel. Panel (E) displays leukocytes exiting the main flow pathway (s6)-(s7) into the $10\ \mu\text{m}$ -wide extraction channel (s8); (F) shows corresponding leukocyte distribution. [Shevkoplyas et al., *Anal. Chem.* 77(3), 933-937, 2005]

[15,16,24]. Briefly, a silicon wafer containing a negative image of the microchannel device was created at the Cornell Nanofabrication Facility using electron beam lithography and reactive ion etching techniques. This master wafer was then used as a negative mold to cast replicas of the device in PDMS. Cast replicas were trimmed to size and affixed onto PDMS-coated glass slides following brief exposure to air plasma. Assembled microdevices were flushed with 1% aqueous solution of mPEG-silane to prevent cell adhesion and then washed with buffer. Whole human blood was collected by venipuncture from healthy consenting volunteers into Vacutainer tubes

containing anticoagulant EDTA similar to the standard clinical procedure. Blood samples were introduced into a feeding reservoir incised in the PDMS replica to provide access to the channels. Blood exited the device through a 2-mm hole drilled in the glass slide and then drained into a waste collection reservoir. A water column was used to provide the operational pressure gradient of $40\ \text{cmH}_2\text{O}$. The microchannel device assembly was mounted in a custom holder on a calibrated motorized microscope stage. Sequences of images were acquired with a CMOS digital camera using a wide bandpass blue-violet filter ($407\pm 52\text{nm}$) to improve contrast. Images were recorded and

then analyzed off-line using custom software to determine leukocyte distributions and the leukocyte-to-erythrocyte ratios in the extraction channel. Initial red and white cell concentrations in whole blood were determined in duplicate using an automated cell counter.

3 RESULTS & DISCUSSION

The microseparation device measures approximately 0.7 x 7 mm, and consists of a simple network of microchannels schematically shown in Figure 1. Flow of blood through the device is driven by a small pressure gradient (~40cmH₂O). The device operates as follows:

Whole blood enters the device through a 70- μ m-wide supply channel. Initially, leukocytes are distributed uniformly across the channel (Fig. 1A). As smaller, more flexible erythrocytes seek the faster flow region in the center of the channel lumen [17], they collide with leukocytes forcing them to migrate towards the sidewalls [13]. Therefore, before the first bifurcation is reached (Fig. 1, b1), the leukocyte distribution across the channel changes considerably, with most of the leukocytes traveling near the channel sidewalls (Fig. 1C).

This phenomenon of leukocyte margination in the supply channel appears more dramatic than that observed in real blood vessels. The depth of all channels comprising the device (10.3 μ m) approximates the average leukocyte diameter (10-12 μ m), such that out-of-the-plane motion of leukocytes is effectively restricted. Due to this pseudo-2D geometry, practically all erythrocyte-leukocyte collisions result in lateral migration of the leukocytes toward the nearest sidewall. In contrast, margination in real blood vessels is less efficient because migration of leukocytes is not constrained to any preferential direction. Therefore, when leukocytes marginate *in vivo*, they approach the vessel wall via a random walk (that reflects collisions with erythrocytes from multiple angles) and arrive at points scattered around the vessel perimeter [13].

The concentration of leukocytes in the blood immediately after entering the supply channel (2100/ μ l, Fig. 1A) is less than half of that in the original whole blood sample (4300/ μ l). This is because erythrocytes and plasma flow more easily than leukocytes from the sample reservoir, effectively lowering leukocyte concentration. This phenomenon likely reflects specific design features of the current sample introduction system.

As leukocytes marginate to the sidewalls, they begin to flow slower than the rest of the blood, because of the shape of the velocity profile in the channel. This results in gradual accumulation of leukocytes traveling adjacent to the sidewalls and leads to the increase of overall leukocyte concentration along the supply channel from 2100/ μ l (Fig. 1A) to 4500/ μ l (Fig. 1C). Thus compensating for the 2-fold reduction of leukocyte concentration caused by dynamic dilution at the entry into the channel (Fig. 1A).

After passing through the first bifurcation (Fig. 1, b1), leukocytes continue to travel near the outside wall of each

daughter channel. These two channels have equivalent asymmetric leukocyte distribution profiles, and either can be used for further processing. The asymmetry in leukocyte distribution in segment 4 causes the majority of the leukocytes to enter segment 6 of the second bifurcation, with very few entering segment 5 (data not shown). This further increases the leukocyte concentration in segment 6 by ~2-fold. The asymmetry persists here, with most leukocytes remaining near the right-hand sidewall.

The main part of the leukocyte enrichment device consists of a 135° bend 6 with a small extraction channel 8. The bend alters the velocity profile causing erythrocytes to move quickly around the inside, bypassing leukocytes that travel more slowly in the plasma-rich region near the sidewall. As the erythrocytes pass the slower leukocytes, they trap them near the wall and propel them into the extraction channel (Fig. 1E). Approximately 67% of all leukocytes from the segment 6 enter the extraction channel 8; the rest continue along the right-hand sidewall of the adjacent, larger channel 7 with a characteristic asymmetric distribution (Fig. 1F). Compared to the initial whole blood sample, the leukocyte concentration in the extraction channel 8 is increased ~10-fold, achieving 42300/ μ l.

Both leukocyte margination and plasma skimming are principally important for the enrichment function of the device: accumulation of leukocytes near the sidewall in the plasma-rich region causes ~2/3 of them to enter the extraction channel; at the same time, plasma skimming reduces the erythrocyte concentration in the extraction channel to less than 1/3 of its initial value. (Note that 'plasma skimming' in this context differs somewhat from its conventional definition due to the high concentration of leukocytes in the plasma rich region.) The combined effect of these phenomena translates into an increase in the leukocyte-to-erythrocyte ratio from 1:1100 in the original blood sample to 1:32 in the extraction channel (Fig. 1, s8) - a 34-fold enrichment.

Any practical microanalytical device for point-of-care use must incorporate a robust and convenient way to prepare a sample directly on the chip to minimize the required raw sample size and potential user-induced errors. Most current prototypes, however, concentrate more on the analysis and quantification steps, rather than integrated sample preparation procedures, either because sample preparation is often the most difficult [18] or least exciting [4] part of the assay [5]. Thus, even though such devices generally require only sub-microliter sample volumes for analysis, off-the-chip sample preparation via conventional centrifugation may require several milliliters of blood.

Currently available approaches to on-the-chip sample preparation are based on filtration [2,18], dielectrophoresis (DEP) [19] or require preliminary cell modification steps such as fluorescent labeling [20,21] or tagging with magnetic beads [22,23]. The current state of the art of these and other sample preparation methods is the focus of recent reviews [1,3,4,5].

The new leukocyte enrichment method described here has many important advantages over these technologies. It is simple, has no active (moving or otherwise) elements, requires only a small hydrostatic pressure gradient to function, and can be easily manufactured using conventional microfabrication. Further, it operates on (anticoagulated) whole blood, actually benefiting from physiological levels of hematocrit – the same factor that confounds other sample preparation techniques. It provides positive selection of leukocytes, which are not trapped in a specific area of the chip (in contrast to both filtration [18] and DEP [19]), but continue to flow and can be easily transported elsewhere on the chip for further purification or directly to analytical unit(s) for examination. Importantly, leukocyte handling (and thus potential leukocyte activation) is minimized and no cell pre-processing is required, as opposed to fluorescence- or magnetically-activated cell separation procedures [20,21,22,23].

The separation is efficient, producing a greater than 30-fold increase in the leukocyte-to-erythrocyte ratio in a single pass. This is higher than that of a single spin centrifugation “buffy coat” preparation of whole human blood (~10-20-fold enrichment) and is sufficient for many applications such as PCR, which requires at least a 10-fold enrichment.

Future work is needed to improve purity and provide better characterization of this new leukocyte enrichment technique. For example, modification of the input reservoir may eliminate the initial dynamic reduction of leukocyte concentration caused by the impeded traffic of leukocytes during their entry into the supply channel (Fig. 1A). In addition, separation units can be arranged in series to further purify the extraction channel effluent (Fig. 1, s8). However, the low hematocrit (~1/3 of initial sample) and high leukocyte concentration (~10 times higher than in initial sample) characteristic of the effluent may diminish the efficiency of secondary separation. Decreased frequency of erythrocyte-leukocyte collisions and increased number leukocyte-leukocyte interactions could hinder leukocyte margination and reduce separation efficiency. Another possible approach aimed at higher separation efficiency would involve coating the supply channel walls with adhesion molecules, such as P-selectin, which mediate leukocyte rolling *in vivo*. This slow ‘adhesive’ rolling of leukocytes would facilitate their accumulation along the supply channel and may improve overall enrichment.

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